

Appl. No. 10/500,878  
Response dated May 14, 2007  
Reply to Office Action of April 12, 2007

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**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

**Claim 1 (withdrawn):** A method to identify a ligand site obeying a two-state or more complex binding behavior in a transient complex of a ligand with a target molecule, said method comprising the steps of:

- a) preparing a ligand with at least one nucleus detectable by NMR;
- b) collecting NMR relaxation dispersion profiles for said nucleus at two or more magnetic fields;
- c) determining apparent transverse relaxation rates from said dispersion profiles of step b);
- d) assigning resonance peaks to said nucleus of the ligand with one- and/or multi-dimensional NMR;
- e) contacting the ligand of step a) with at least one concentration of a target molecule;
- f) for each contacting of said ligand with at least one concentration of said target molecule as defined in step e) collecting NMR relaxation dispersion profiles for said ligand contacted with said at least one concentration of said target molecule for every concentration of said target molecule at two or more magnetic fields;
- g) fitting said dispersion profiles obtained in step f) by including the relaxation rates of step c) and using a two-state exchange model independently for every nucleus, and independently or simultaneously for every concentration of the target molecule; and
- h) determining a ligand site obeying a two-state binding behavior based on feasibility of extracted  $R_{2b}$  and  $p_b$  parameters obtained by the fitting of step g), wherein  $R_{2b}$  represents a nominal transverse relaxation rate of said ligand bound to the target molecule and  $p_b$  represents a nominal fraction of the bound ligand.

**Claim 2 (withdrawn):** The method of claim 1, wherein the ligand of step a) has at least two detectable nuclei.

**Claim 3 (withdrawn):** The method of claim 1 or 2, wherein the ligand in step e) is contacted with at least two concentrations of a target molecule.

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Claim 4 (withdrawn): The method of claim 3, wherein the ligand is contacted with three concentrations of target molecules.

Claim 5 (currently amended): A method to determine quantitatively the dissociation rate constant ( $k_{off}$ ) for a transient complex of a ligand with the target molecule comprising the steps of:

a) ~~Identifying~~ identifying a ligand site obeying a two-state binding behavior in a transient complex of a ligand with a target molecule ~~with the method as defined in claim 1, 2, 3 or 4 by~~

i) preparing a ligand with at least one nucleus detectable by NMR;

ii) collecting NMR relaxation dispersion profiles for said nucleus at two or more magnetic fields;

iii) determining apparent transverse relaxation rates from said dispersion profiles of step ii);

iv) assigning resonance peaks to said nucleus of the ligand with one- and/or multi-dimensional NMR;

v) contacting the ligand of step i) with at least one concentration of a target molecule;

vi) for each contacting of said ligand with at least one concentration of said target molecule as defined in step v) collecting NMR relaxation dispersion profiles for said ligand contacted with said at least one concentration of said target molecule for every concentration of said target molecule at two or more magnetic fields;

vii) fitting said dispersion profiles obtained in step vi) by including the relaxation rates of step iii) and using a two-state exchange model independently for every nucleus, and independently or simultaneously for every concentration of the target molecule; and

viii) determining a ligand site obeying a two-state binding behavior based on feasibility of extracted  $R_{2b}$  and  $p_b$  parameters obtained by the fitting of step vii), wherein  $R_{2b}$  represents a nominal transverse relaxation rate of said ligand bound to the target molecule and  $p_b$  represents a nominal fraction of the bound ligand; and

b) ~~Extracting~~ extracting  $k_{off}$  values for the ligand sites obeying two-site exchange mechanism, said off values being a measure of the affinity of a transient complex of the ligand with the target molecule.

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Claim 6 (currently amended): A method according to claim ~~1, 2, 3, 4, or 5~~, wherein the ligand is a polypeptide.

Claim 7 (currently amended): A method according to claim ~~1, 2, 3, 4, 5 or 6~~, wherein the ligand is a <sup>15</sup>N-enriched polypeptide.

Claim 8 (currently amended): A method according to claim ~~1, 2, 3, 4, 5, 6 or 7~~, wherein the ligand is a mixture of polypeptides and/or molecules.

Claim 9 (currently amended): A method according to claim ~~1, 2, 3, 4, 5, 6, 7 or 8~~, wherein the target molecule is a protein or a protein assembly.

Claim 10 (withdrawn): Use of the method as defined in claim 1, 2, 3, 4, 5, 6, 7, 8 or 9 to determine amino acid residues with detectable NMR relaxation dispersion as constituting binding hot-spots.

Claim 11 (withdrawn): Use of the method as defined in claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 to identify two or more ligands that can be linked together to create high-affinity molecules.

Claim 12 (withdrawn): Use of the method of claim 1, 2, 3, 4, 5, 6, 7, 8 or 9 to study high-affinity protein-protein interactions or slow-dissociating ligand-target complexes.

Claim 13 (withdrawn): The use of claim 10, 11 or 12, wherein the ligand is a polypeptide or a protein.

Claim 14 (withdrawn): The use of claim 10, 11, 12 or 13, wherein the ligand is <sup>15</sup>N-enriched.

Claim 15 (currently amended): The method of claim 5, wherein any one of claims 1 to 9, where the NMR relaxation dispersion profiles are collected by a CPMG method.

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Claim 16 (withdrawn): The use of claims 10 to 14 where the NMR relaxation dispersion profiles are collected by a CPMG method.

Claim 17 (new): The method of claim 5, wherein the ligand of step i) has at least two detectable nuclei.

Claim 18 (new): The method of claim 5, wherein the ligand in step v) is contacted with at least two concentrations of a target molecule.

Claim 19 (new): The method of claim 5, wherein the ligand is contacted with three concentrations of target molecules.